



# A Summary of Eelgrass (*Zostera marina*) Reproductive Biology with an Emphasis on Seed Biology and Ecology from the Chesapeake Bay Region

by Robert J. Orth, Scott R. Marion, and Kenneth A. Moore

**BACKGROUND:** Interest in seagrass restoration is increasing worldwide as the value of seagrass ecosystems is recognized by scientists, managers and regulators (Orth et al. 2000, 2006b, 2006c). Seagrass transplanting projects have traditionally relied on adult plants (Fonseca et al. 1998) using a variety of manual and mechanical techniques (Fonseca et al. 1998, Fishman et al. 2004, Treat and Lewis 2006). However, most techniques using adult plants are labor-intensive and time-consuming, requiring physical excavation of the donor material, which could be deleterious to the donor bed's survival. In addition, transporting adult plants can present logistical constraints if the transplant site is located at significant distances from the donor site, or if the methodology requires moving sediment along with the plants. One of the key advantages of transplanting adult plants is the immediate creation of habitat for fauna, which have been shown to colonize these areas rapidly (Fonseca et al. 1996).



Figure 1. Eelgrass (*Zostera marina*).

While most seagrass restoration projects cover small areas of meters to tens of meters squared, efforts to restore larger areas may be necessary to significantly enhance recovery. Seeds offer the potential to restore large, genetically diverse populations of submerged aquatic vegetation (SAV) in a manner that avoids the damage to donor beds caused by harvesting adult transplants. Seagrass seeds have been shown to be critical in natural bed recovery following disturbances (Plus et al. 2003) and in initiating recovery in systems where seed recruitment is rare (Orth et al. 2006d). Much of the research on the use of seeds in SAV restoration has focused on a single species, *Zostera marina* (eelgrass) (Figure 1). As a result, more is known about the seed ecology of *Z. marina* than any other seagrass species. This knowledge has been successfully applied to initiate recovery of eelgrass beds in the Virginia seaside coastal lagoons that were decimated by the 1930s 'wasting disease' pandemic (Orth et al. 2006d). Although recent

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research has expanded understanding of eelgrass seed biology and ecology and its application in SAV restoration, numerous challenges remain in order to increase the success of eelgrass planting from seed.

**PURPOSE:** This technical note summarizes the status of current knowledge of eelgrass (*Zostera marina*) reproductive biology in the Chesapeake Bay region, with an emphasis on seed biology and ecology and the relevance of this information in restoring eelgrass.

**BASIC REPRODUCTIVE BIOLOGY:** The basic reproductive biology of eelgrass in the Chesapeake Bay region relevant to restoration can be summarized as follows:

1. Eelgrass has a bimodal cycle of clonal growth, which is closely linked to water temperature (Moore 1992, Moore et al. 1996). Peak biomass and shoot density occur in late spring as water temperatures rise to 25 °C. During the summer months, when water temperatures rise above 25 °C and may exceed 30 °C in July and August, shoot production declines rapidly and leaf senescence occurs. Large mats of floating eelgrass leaves occur in July and are deposited on shorelines as wrack. Shoot production commences again in mid-September as temperatures drop below 25 °C and generally continues through the fall until water temperatures drop below 10 °C (Orth and Moore 1986, Moore 1992).
2. Flowering is initiated during the winter months. Flowering shoots are sometimes readily noted as early as February, with anthesis occurring in April, and mature seeds being released from mid-May to early June (Silberhorn et al. 1983).
3. Flowering shoot densities are highly variable in the Chesapeake Bay region, generally comprising 10-20 percent of the total number of shoots per square meter (Orth and Moore 1986; unpublished data). Leaf lengths of flowering shoots are also highly variable and range from 25-30 cm in some very shallow-water areas to 1.0 -1.5 m, generally in deeper water depths and in areas with sediments with higher silt-clay and organic content.<sup>1</sup>
4. Seed production is a function of number of spathes per rhipidia (rhipidia = group of inflorescences), number of rhipidia per flowering shoot, and number of flowering shoots per unit area (Silberhorn et al. 1983), and can range from millions to hundreds of millions of seeds per acre.<sup>1</sup>
5. Seed germination occurs in the fall around November as temperatures drop below 15 °C. Germination is cued by an interaction of temperature and lack of oxygen (Moore et al. 1993).
6. Eelgrass has a short-lived, transient seed bank of approximately 5 months, with a maximum of up to 10 months (Orth and Moore 1983, Moore et al. 1993, Harwell and Orth 2002a). There is no substantial evidence of seeds surviving more than one year in the seed bank.

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<sup>1</sup> Unpublished data, Robert J. Orth, Department of Biological Sciences, Virginia Institute of Marine Science, School of Marine Science, College of William and Mary, Gloucester Pt., VA.

7. Flowering shoots are typically produced in the second year of a plant's growth cycle following seed germination (Setchell 1929). No annual populations have been reported in this region, although on rare occasions a few seedlings with flowering shoots have been noted in late spring.<sup>1</sup>

**EELGRASS SEED BIOLOGY AND ECOLOGY:** Recently, seeds have been shown to be important in creation of new patches, recovery of beds lost due to disturbance, and in providing genetic diversity (Plus et al. 2003, Orth et al. 2006c), suggesting seagrass seeds could play an important role in seagrass restoration efforts (Orth et al. 2000, 2006c). Research investigating eelgrass seed biology and ecology in this region has revealed the following:

1. Reproductive shoots have biomechanical properties that allow some shoots to be resistant to being ripped out in extreme events, resulting in seed retention within a bed (Patterson et al. 2001), while other whole shoots or rhipidia can be dislodged and dispersed from the bed.
2. Reproductive shoots with viable, mature seeds, when broken off in the late spring (May and early June), can float distances of 100 km or greater (Harwell and Orth 2002b) (Figure 2).
3. Healthy, viable seeds settle rapidly (mean settling velocity  $4-6 \text{ cm sec}^{-1}$ ) and generally do not move far from where they are deposited onto the sediment surface, becoming rapidly incorporated into the sediment (Orth et al. 1994).
4. Small-scale topographic features on the bottom such as animal burrows, pits, and mounds, act to shield seeds from flow and prevent their being washed out (Luckenbach and Orth 1999).
5. Seeds need to be incorporated into the sediment to facilitate germination (Moore et al. 1993).
6. Blue crabs, as well as other decapods, have been shown to be important seed predators (Wigand and Churchill 1988, Fishman and Orth 1996).<sup>2</sup>
7. Eelgrass reproductive shoots with viable seeds that are drifting along the bottom can be captured by an infaunal, tube-building polychaete (*Diopatra cuprea*). The shoot is then "cemented" into the tube cap along with shells and macroalgae. Seeds from these retained shoots are then released as the shoot decays, remain near the *Diopatra* tube, and develop into viable plants (Harwell and Orth 2001).



Figure 2. Detached eelgrass reproductive shoot.

<sup>1</sup> Personal observation, Robert J. Orth, Department of Biological Sciences, Virginia Institute of Marine Science, School of Marine Science, College of William and Mary, Gloucester Pt., VA.

<sup>2</sup> Unpublished data, Robert J. Orth, Department of Biological Sciences, Virginia Institute of Marine Science, School of Marine Science, College of William and Mary, Gloucester Pt., VA.

8. Germination and initial seedling establishment do not appear to be density dependent, i.e., more seeds mean more plants (Orth et al. 2003). However, when seeds are broadcast at high densities (e.g.,  $>1,000 \text{ m}^{-2}$ ), seedlings observed the following spring are clumped and their individual growth is much less than growth of an isolated seedling. Survivorship of seedlings growing initially in dense clumps may also be low. VIMS scientists have noted a difference in growth form of seedlings growing individually (much bigger) versus those in clumps (small plants). However, no longer-term data are available, as the vigorous growth of these plants after the first year compromises precise estimates of shoot density per seedling. Data from Granger et al. (2000) showed seedlings from plots sowed at lower densities (500-1000  $\text{m}^{-2}$ ) had significantly more lateral shoots than seedlings in plots sowed at higher densities (2000-4000  $\text{m}^{-2}$ ).
9. Seeds appear to be generally more important in establishing new patches distant from established beds (Harwell and Orth 2002b), or in areas where eelgrass has been disturbed,<sup>1</sup> than in maintaining the structure of established beds.
10. Seedling growth is initially slow during winter months, but increases rapidly in the spring and can result in a single plant with up to 25-30 shoots that occupies an area of up to 0.25  $\text{m}^2$  (Orth and Moore 1983).<sup>1</sup> While an earlier report suggests that seedlings have habitat requirements similar to adult plants and respond similarly to nutrient enrichment (Roberts et al. 1984), recent data indicate that seedlings may have a lower lethal temperature tolerance (i.e., during the summer of 2005, seedlings at major restoration sites in the coastal bays all died while older plants survived).<sup>1</sup>

### **LESSONS LEARNED FROM EELGRASS RESTORATION USING ADULT PLANTS:**

Much of the earliest restoration work in Chesapeake Bay was conducted in a variety of locations with different vegetation histories and water quality characteristics to facilitate addressing questions related to growth and habitat requirements (Moore 1992; Moore et al. 1996, 1997). Techniques for transplanting initially emphasized adult plants, with subsequent emphasis on seeds. Early restoration efforts with adult plants showed:

1. Planting eelgrass plants in fall rather than spring was optimal because plants had a longer growing period to become established before the summer stress period (Orth et al. 2006a).
2. Water quality conditions during the growing season strongly affect restoration success. Poor water clarity for periods as short as 2-3 weeks during the growing season may result in complete restoration failure (Moore et al. 1996, 1997).
3. Addition of fertilizer to transplants increased plant density but did not enhance long-term survival (Orth and Moore 1982, Orth et al. 2006a).
4. Techniques utilizing adult plants (e.g., mesh mats with bare-rooted shoots, sods and cores of seagrass and sediment, bundles of bare-root shoots with anchors, single shoots without anchors) were generally successful, with the manually planted single shoot method generally being both successful and requiring the least time (Orth et al. 1999, 2006a).

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<sup>1</sup> Ongoing and unpublished data, Robert J. Orth, Department of Biological Sciences, Virginia Institute of Marine Science, School of Marine Science, College of William and Mary, Gloucester Pt., VA.

5. Mechanized planting of adult plants with a planting boat demonstrated that while the planting boat was capable of planting more rapidly than manual methods, this potential advantage was offset by reduced initial planting success, resulting in a greater cost per surviving planting unit after 24 weeks compared to manual methods (Fishman et al. 2004).

**LESSONS LEARNED FROM EELGRASS RESTORATION USING SEEDS:** Transplant projects incorporating seeds have been relatively rare despite the fact that some seagrasses produce large quantities of seeds, ranging up to tens of thousands per square meter (Orth et al. 2006c). Seed-based eelgrass restoration, seed ecology, and conservation have recently focused on harvesting, seed storage techniques, seed broadcast timing and techniques, and understanding the relative importance of seeds in either bed persistence or in development of new beds. Experiments conducted using eelgrass seeds over the past two decades have shown the following:

1. Seed production can be temporally and spatially variable (Silberhorn et al. 1983, Orth and Moore 1986) and may require weekly assessments of multiple sites to determine which areas will be most suitable for harvesting reproductive shoots that would yield the highest quantity of seeds per collecting hour.<sup>1</sup>
2. Weather conditions play a major role in facilitating the harvesting of shoots. Wind can compromise water clarity for efficient hand harvesting and create unsafe conditions for mechanical harvesting.<sup>1</sup>
3. Optimum collection periods typically last only 1-2 weeks for individual locations. Different sites in a region may vary up to several weeks in reproductive development. Cooler than normal water temperatures during the spring may delay the development of mature seeds for up to several weeks. Conversely, above-normal water temperatures during the spring may accelerate seed development and release.
4. Harvesting flowering shoots by hand is labor-intensive and limited by weather and water quality conditions, and in some situations may require SCUBA. However, if storage space is limited, hand harvesting may be the best option for producing the largest number of seeds (Figure 3).
5. Mechanical harvesting of reproductive shoots has proven successful (Figure 4), but its use in a particular region should be evaluated based on reproductive shoot densities, storage capabilities, and size of donor beds available for harvesting (Orth and Marion 2007).



Figure 3. Diver collecting eelgrass reproductive shoots.

<sup>1</sup> Unpublished data and personal observations, Robert J. Orth, Department of Biological Sciences, Virginia Institute of Marine Science, School of Marine Science, College of William and Mary, Gloucester Pt., VA.

6. Mechanical harvesting results in a large volume of vegetative material, which requires large tanks and high seawater delivery capacity, which could be expensive to maintain. Processing, if done manually, could be lengthy. Storage conditions while seeds are being released in the holding tanks must be monitored for appropriate conditions such as salinity, water turnover, and temperature (Orth and Marion 2007). Care should be taken to remove blue crabs from the storage tanks. These decapods occur as by-catch in machine-harvested material and could eat many seeds in the tanks during the seed release period (Fishman and Orth 1996).<sup>1</sup> Blue crabs do not appear in hand-harvested stock.
7. Following separation of seeds from the large holding tanks, optimal storage in the Chesapeake Bay region involves removing as much organic matter as possible, and maintaining the seeds in a high salinity (20–30 psu) recirculating system at moderate temperatures (<24 °C) (Orth and Marion 2007).
8. Techniques for incorporating seeds in both small- and large-scale restoration projects (e.g., peat pots, seed broadcasting, and burlap bags to protect seeds) have had varying degrees of success. The highest seedling establishment has been noted where seeds were protected in burlap bags (Harwell and Orth 1999, Orth et al. 2006a).
9. Experimental field-based tests of seed germination in late spring, summer, and fall revealed higher establishment rates for seeds broadcast in the fall.<sup>1</sup>
10. A recently developed system of deploying flowering shoots when first harvested in floating mesh bags (e.g. Buoy-Deployed Seeding System) has resulted in successful seedling establishment (Pickerell et al. 2005, 2006).<sup>1</sup> The method offers the advantages of not requiring expensive holding systems and simple and inexpensive construction (Figure 5). However, experience with large-scale deployments (>1,000 units) revealed a number of logistical constraints. These included: a) obtaining permits to deploy floating bags in navigable waters, b) the handling of a significant volume of material (including both vegetative and reproductive material) and sheer number of individual units including the



Figure 4. Mechanical harvester.



Figure 5. Buoy-deployed seeding technique.

<sup>1</sup> Unpublished data, Robert J. Orth, Department of Biological Sciences, Virginia Institute of Marine Science, School of Marine Science, College of William and Mary, Gloucester Pt., VA.

weight of the holding blocks, and c) the physical removal of the units, made more difficult at some sites by accumulation of macroalgae on the bags. In addition, a comparison of seeds deployed with these bags versus broadcasting revealed a lower rate of seedling establishment as seeds were placed in the spring and exposed to field-based mortality factors for 5 months longer.<sup>1</sup>

11. Broadcasting seeds in the fall prior to seed germination is one of the least labor-intensive techniques used to date and is currently proving successful in restoring eelgrass to Virginia's seaside coastal bays that have been unvegetated since the 1930s pandemic wasting disease (Orth et al. 2006d) (Figure 6).
12. Seeds have been broadcast at densities ranging from 2 to 1,250 seeds/m<sup>2</sup> in small-scale plots. Large-scale restoration has been successful at seed densities of 100,000–200,000 seeds per acre or 25–50 seeds/m<sup>2</sup> (Orth et al. 2006d), and in some locations lower densities (12.5 seeds/m<sup>2</sup>) have eventually formed successful plots over a longer time frame.
13. While new and innovative techniques for planting seeds are emerging, it appears that the biggest effect is location, suggesting that for each area, different techniques should be investigated for best results (Orth et al. 2003).
14. Comparison of seedling establishment rates using a seed planting machine (Figure 7) (Traber et al. 2003) with our traditional hand broadcasting showed promise for increasing seedling establishment relative to seed broadcasting, but its effectiveness varied among the three restoration sites tested (Orth et al., in preparation).
15. The most significant bottleneck in seed-based restoration projects remains the low initial establishment rate of seeds (generally between 1 and 10 percent of seeds establishing as



Figure 6. Aerial view of eelgrass plots established using broadcast seeding.

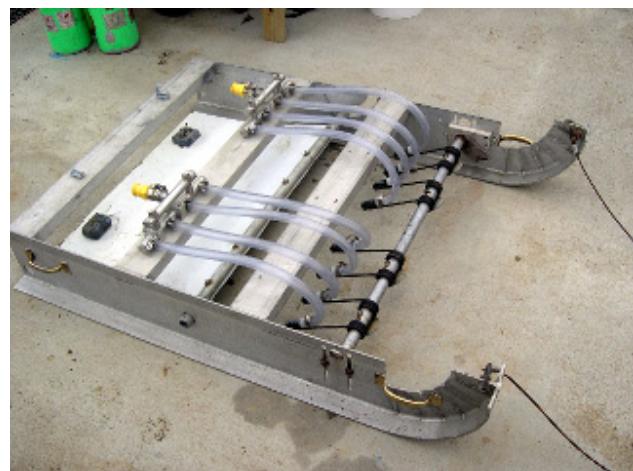


Figure 7. Underwater mechanical seed planter.

<sup>1</sup> Unpublished data, Robert J. Orth, Department of Biological Sciences, Virginia Institute of Marine Science, School of Marine Science, College of William and Mary, Gloucester Pt., VA.

seedlings in field experiments) (Orth et al. 2006d). Restoration efforts have been successful even with this limitation, but achieving greater initial establishment would allow the same limited stock of seeds to be spread across a greater area, increasing the number of sites and reducing the cost per restored acre.

**SUMMARY AND RECOMMENDATIONS:** A significant amount of knowledge has accumulated over the years on eelgrass reproductive biology, which has proven quite informative in evaluating restoration techniques and processes in the Chesapeake Bay region. The authors believe that much of this knowledge is relevant to eelgrass restoration projects both here and abroad. Listed below are the most significant aspects of the findings to date for this region:

1. Multiple methods of transplanting adult plants have proven successful, but are considered less efficient than seed-based methods. If planting adults is required, transplanting should be done in the fall when eelgrass commences its fall growth period and water temperatures are between 15 and 25 °C.
2. Seed-based restoration is efficient if the target site is conducive to seedling establishment, as large numbers of seeds can be easily harvested in most areas and stored until broadcasting later the same year.
3. If seeds are stored, they should be placed in recirculating seawater tanks with salinities between 20 and 30 psu, and at temperatures between 15 and 24 °C.
4. Loss of seed in the field can be reduced by broadcasting in the fall in October just prior to seed germination.
5. Broadcasting seeds has proven successful, but low seedling establishment rates ultimately constrain the area that can be restored.

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